

## PATENT COOPERATION TREATY

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

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EINGANG / RECEIPT  
29.03.2004

NOTIFICATION OF TRANSMITTAL OF  
THE INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT  
(PCT Rule 71.1)

Erl.: .....

Date of mailing

(day/month/year)

26.03.2004

Applicant's or agent's file reference

Case 21146

## IMPORTANT NOTIFICATION

International application No.

PCT/EP 03/01473

International filing date (day/month/year)

14.02.2003

Priority date (day/month/year)

22.02.2002

Applicant

DSM IP ASSETS B.V. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

## 4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international  
preliminary examining authority:



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**PATENT COOPERATION TREATY**  
**PCT**  
**INTERNATIONAL PRELIMINARY EXAMINATION REPORT**  
(PCT Article 36 and Rule 70)



REC'D 29 MAR 2004

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Applicant's or agent's file reference Case 21146	<b>FOR FURTHER ACTION</b>	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)
International application No. PCT/EP 03/01473	International filing date (day/month/year) 14.02.2003	Priority date (day/month/year) 22.02.2002
International Patent Classification (IPC) or both national classification and IPC C12N9/02		
Applicant DSM IP ASSETS B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
- ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).
- These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:
- I ☒ Basis of the opinion
  - II ☐ Priority
  - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
  - IV ☐ Lack of unity of invention
  - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
  - VI ☐ Certain documents cited
  - VII ☐ Certain defects in the international application
  - VIII ☐ Certain observations on the international application

Date of submission of the demand  24.07.2003	Date of completion of this report  26.03.2004
Name and mailing address of the international preliminary examining authority:   European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer  Donath, C  Telephone No. +49 89 2399-8710  

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. **PCT/EP 03/01473**

**I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, Pages**

1-12 as originally filed

**Claims, Numbers**

1-10 received on 23.02.2004 with letter of 20.02.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.  
☐ filed together with the international application in computer readable form.  
☐ furnished subsequently to this Authority in written form.  
☐ furnished subsequently to this Authority in computer readable form.  
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☒ the claims, Nos.: 11  
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

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International application No. **PCT/EP 03/01473**

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**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;  
citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes: Claims	1-6
	No: Claims	7-10
Inventive step (IS)	Yes: Claims	1-6
	No: Claims	7-10
Industrial applicability (IA)	Yes: Claims	1-10
	No: Claims	

**2. Citations and explanations**

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP03/01473

**Ad section V.:**

1. The following document is cited:

D1 EP 1 074 630

D2 Eur.J.Biochem. 255, 271-278, 1998

2. The present International application refers to an isolated enone reductase characterized by several physico-chemical properties, a process for producing said enone reductase and a process for producing levodione from ketoisophorone by use of said enone reductase or by use of an microorganism capable of producing said enzyme.

In view of the documents cited in the International Search Report only the subject-matter of claims 1-6 of the present International application has to be regarded as being new (Article 33(2) PCT).

- 2.1 D1 describes a process for producing levodione by use of different yeast strains which are shown to be capable of converting ketoisophorone into levodione. The reaction for producing levodione was carried out at a temperature range from 20 to 40°C and at a pH from 3.0 to 6.0. The strain giving the best results was *Zygosaccharomyces rouxii*, however, it was shown that as well other yeasts such as *Saccharomyces* and *Candida* strains have the ability to convert ketoisophorone into levodione and thus, can be used in the above process. (see D1, p.2, line 20 - p.3, line 48, examples 1-3).

According to the description the enone reductase of the present International application can be obtained from yeast e.g. *Zygosaccharomyces rouxii*, or in the process for producing levodione cells or cell-free extract of a microorganism belonging to the genus *Candida* are used.

Therefore, the disclosure of D1 is novelty-destroying for the subject-matter of claims 7 - 10 insofar as the process comprises contacting ketoisophorone with cells or a cell-free extract of a microorganism belonging to the genus *Candida* capable of producing the enone reductase.

3. The closest prior art to evaluate the inventiveness of the subject-matter of the present International application is D2.  
D2 discloses the purification and characterization of two enone reductases from

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/EP03/01473

*Saccharomyces cerevisiae*. One enzyme was NADPH-dependent, showed a molecular mass of 75 kDa and decomposed under denaturing electrophoretic conditions into two subunits of 34 kDa and 37 kDa. The other enzyme was NADH linked and the molecular mass estimated by means of Superdex 200 preparation-grade filtration was 130 kDa. the enzyme decomposed into two subunits of 56 kDa and 64 kDa on SDS/PAGE. Both enzymes were most active at pH 4.8 and the temperature optima of the enzymes have been found between 30-38°C. Neither in D2 nor in any other of the cited documents of the International Search Report an indication has been given for an isolated enone reductase having the physico-chemical properties of the enzyme claimed in the present International application.

Thus, an inventive step has to be acknowledged for claims 1-6 of the present International application (Article 33(3) PCT).

Claims

1. An isolated enone reductase having the following physico-chemical properties:

- a) Molecular mass:  $61,300 \pm 5,000$  Da  
(Consisting of one subunit having a molecular mass of  $45,000 \pm 5,000$  Da),
- b) Co-factor: NADPH and NADH,
- c) Substrate specificity: active on  $\alpha$ ,  $\beta$ -unsaturated ketones,
- d) Optimum temperature: 55 - 60°C at pH 7.4,
- e) Optimum pH: 4.5 - 8.5.

2. An enone reductase according to claim 1, which is derived from a microorganism which is capable of producing the enone reductase having the properties as defined in claim 1.

3. An enone reductase according to claim 2, wherein the microorganism is a yeast.

4. An enone reductase according to claim 2, wherein the microorganism is *Candida kefyr* (*Candida macedoniensis*) IFO 0960, its functional equivalents, subcultures, mutants or variants.

5. A process for producing an enone reductase having the following physico-chemical properties:

- a) Molecular mass:  $61,300 \pm 5,000$  Da  
(Consisting of one subunit having a molecular mass of  $45,000 \pm 5,000$  Da),
- b) Co-factor: NADPH and NADH,
- c) Substrate specificity: active on  $\alpha$ ,  $\beta$ -unsaturated ketones,
- d) Optimum temperature: 55 - 60°C at pH 7.4,
- e) Optimum pH: 4.5 - 8.5.

which process comprises cultivating a microorganism, which is capable of

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producing the enone reductase having the above properties, in an aqueous nutrient medium under aerobic conditions, disrupting the cells of the microorganism, and isolating and purifying the enone reductase from said extract.

6. The process according to claim 5, wherein the microorganism is a yeast.

7. A process for producing levodione from ketoisophorone which process comprises contacting ketoisophorone with

(i) an enone reductase having the following physico-chemical properties:

a) Molecular mass:  $61,300 \pm 5,000$  Da  
(Consisting of one subunit having a molecular mass of  $45,000 \pm 5,000$

Da),

b) Co-factor: NADPH and NADH,

c) Substrate specificity: active on  $\alpha$ ,  $\beta$ -unsaturated ketones,

d) Optimum temperature:  $55 - 60^\circ\text{C}$  at pH 7.4,

e) Optimum pH: 4.5 - 8.5.

in the presence of NADH or NADPH; or

(ii) cells or a cell-free extract of a microorganism belonging to the genus Candida, capable of producing the enzyme as defined in (i),

and isolating the resulting levodione from the reaction mixture.

8. The process according to claim 7, wherein the microorganism is a yeast.

9. The process according to claims 7 or 8, wherein the reaction is conducted at a pH in the range of from 4.5 to 8.5.

10. The process according to any one of claims 7 to 9, wherein the temperature of the reaction is in the range of from  $30$  to  $60^\circ\text{C}$ .

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